Flex et al., http://www.jem.org/cgi/content/full/jem.20072182/DC1

SUPPLEMENTAL MATERIALS AND METHODS

Molecular modeling. Structural models of the kinase, pseudokinase, SH2, and FERM domains of JAK1 were obtained by employing DeepView software and the Swiss Model server (1, 2) after manual optimization of the alignments. The kinase domain (residues 876–1,153) was modeled by homology to the crystallographic structures of the following kinase domains obtained from the Protein Data Bank (pdb): JAK2 (pdb code 2b7a, identity 54%), JAK3 (pdb code 1yvj, identity 51%), FGFR1 (pdb code 1fgi, identity 35%), and FGFR2 (pdb code 1oec, identity 34%). Residues 604–852 of the pseudokinase domain (residues 583–855) were modeled by homology to the structures of the kinase domains of RET (pdb code 2ivt, identity 26%) and ABL (pdb code 1fpu, identity 24%). The SH2 domain (residues 439–544) was modeled by homology to the structures of the following SH2 domains: GRB10 (pdb code 1nrv, identity 23%), LCK (pdb code 1lkk, identity 21%), HCK (pdb code 1qcf, identity 20%), and SHP-2 (pdb code 2shp, C-terminal SH2 domain, identity 16%). The FERM domain (residues 34–420) was modeled by homology to the structures of the FERM domains of the following proteins: ezrin (pdb code 1ni2, identity 12%), radixin (pdb code 1j19, identity 14%), moesin (pdb code 1e5w, identity 12%), and focal adhesion kinase (pdb code 2al6, identity 15%). A possible relative orientation of the four domains was obtained by superimposing them on a complete model of JAK2 (3). Despite the fact that the modeled JAK1 and JAK2 structures were obtained with different protocols and using different substrates for homology modeling, conformations of JAK1 and JAK2 domains were well superimposed, with Cα root mean square deviations of 1.1 Å (kinase), 1.2 Å (pseudokinase), 1.3 Å (SH2), and 1.6 Å (FERM), supporting the reliability of both models. Molecular graphics were created with the program MOLMOL (4).

Gene expression profiling. Thawed or freshly isolated cells (>90% blasts) were homogenized, and total RNA was extracted either using the Trizol reagent (Life Technologies) and further purified with the SV total isolation system (Promega) or using the RNeasy mini kit (Qiagen). RNA quality was checked by agarose gel electrophoresis and spectrophotometry. HGU133 Plus 2.0 gene chips (Affymetrix) were used to determine gene expression profiles. The detailed protocol for sample preparation and microarray processing is available on the manufacturer's website (http://www.affymetrix.com/support/technical/manual/expression_manual.affx). Oligonucleotide microarray analysis and gene expression data were performed using the dChip software (www.dchip.org) (5), which utilizes an invariant set normalization method where the array with median overall intensity is chosen as the baseline for normalization. Model-based expressions were computed for each array and probe set using only perfect match probes. For unsupervised analysis, nonspecific criteria included the requirement for individual gene expression levels to be >100 in at least 20% of samples, and for the ratio of SD to the mean expression across samples to be included between 0.8 and 1,000. Analysis of variance with P value <0.001 was performed to compare profiles obtained from T-ALL patients with or without a *JAK1* mutation.

REFERENCES

1. Guex, N., and M.C. Peitsch. 1997. SWISS-MODEL and the Swiss-Pdbviewer: an environment for comparative protein modelling. *Electrophoresis*. 18:2714–2723. doi:10.1002/elps.1150181505
2. Schwede, T., J. Kopp, N. Guex, and M.C. Peitsch. 2003. SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res*. 31:3381–3385. doi:10.1093/nar/gkg520
3. Giordanetto, F., and R.T. Kroemer. 2002. Prediction of the structure of human Janus kinase 2 (JAK2) comprising JAK homology domains 1 though 7. *Protein Eng*. 15:727–737. doi:10.1093/protein/15.9.727
4. Koradi, R., M. Billeter, and K. Wüthrich. 1996. MOLMOL: a program for display and analysis of macromolecular structures. *J. Mol. Graph*. 14:51–55. doi:10.1016/0263-7855(96)00009-4
5. Li, C., and W.H. Wong. 2001. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proc. Natl. Acad. Sci. USA*. 98:31–36. doi:10.1073/pnas.011404098